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Psychosocial factors associated with indices of cortisol production in women with breast cancer and controls

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Early morning peak;
Diurnal rhythm;
Breast cancer

Summary The present study was designed to (i) explore which psychosocial factors were associated with indices representing the early morning peak, diurnal cortisol rhythm and area under the curve (AUC); (ii) examine whether the relationships between psychosocial functioning and these cortisol indices were consistent and (iii) explore whether these relationships were influenced by the clinical status of the participant. Newly diagnosed breast cancer patients ($n=85$) and healthy control women ($n=59$) were recruited. State and trait measures of psychosocial functioning (i.e. anxiety, depression, distress, neuroticism, extraversion, marital satisfaction and mastery) were undertaken. In addition, all participants provided four saliva samples (on waking, 30 min later, between 11 and 1 p.m., before lunch and between 8 and 10 p.m., at least 2 h after evening meal) over two consecutive days to assess cortisol levels. The results highlighted the divergent nature of the four cortisol indices; revealed the presence of some significant relationships between the psychosocial measures and the cortisol indices; but highlighted inconsistencies in the relationships evident for patients and those observed for control women.

1. Introduction

The hypothalamic pituitary adrenal (HPA) axis is widely accepted as one of the primary biological pathways through which psychological factors affect the immune system (Haddad et al., 2002;

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Webster et al., 2002). As a consequence, the hormone cortisol, the main effector hormone of this axis in humans, has been the subject of considerable research enquiry. This research effort has, however, been characterised by (i) heterogeneity in the approaches taken to measuring cortisol and (ii) a lack of clarity regarding the relationship between these cortisol indices and measures of psychological functioning and/or clinical outcomes. The present study was designed to capture several measures of cortisol production in women diagnosed with breast cancer and a control population; and to examine the relationship between these cortisol indices and both state and trait measures of psychological functioning.

1.1. Approaches to cortisol measurement

Early studies often reported data involving the measurement of cortisol at a single point in time (Rotton et al., 1997; Brand, 1999). However, the fact that levels of the hormone are affected by a wide range of factors (such as time of day, medication, food in-take, awakening time, etc.: Kirschbaum et al., 1995; Smyth et al., 1997; Federenko et al., 2004) and the propensity for considerable variability in cortisol levels both between and within individuals (Bohnen et al., 1991; Cummins and Gevirtz, 1993), soon led to the measurement of multiple samples, often taken over more than 1 day. Whilst there is little doubt that the measurement of multiple samples has resulted in greater precision, it has also spawned the use of a wide range of approaches to expressing cortisol levels (e.g. area under the curve (AUC), change scores, etc.: Kapuku et al., 2002; Pruessner et al., 2003a; Vedhara et al., 2003). Of these, the calculation of AUC has been adopted most widely in studies involving repeated measures datasets. The AUC is derived from the trapezoid formula, and is typically used in endocrinology to determine the overall secretion of a hormone over a particular time frame. However, more recently, it has been suggested that repeated measures datasets contain information on more than one aspect of the data and that variations in the trapezoid formula can be used to capture these differing components. For example, Pruessner and colleagues (2003a) have suggested that investigators working in the area are likely to be interested in not only total hormone output (as might be captured by conventional AUC formulas), but also the reactivity the system. Thus, they have proposed two different formulas to capture total hormonal output or basal activity of the HPA axis (which they refer to as 'AUC with respect to ground', i.e. AUC_g) and reactivity of the HPA axis (which they refer to as 'AUC with respect to increase', i.e. AUC_i).

In addition to these indices, there is also growing interest in determining measures of the early morning peak and the diurnal pattern of cortisol production. The early morning peak typically involves the measurement of cortisol on waking and again at one or more intervals 15–60 min later (Pruessner et al., 1997). Cortisol levels are then expressed in terms of absolute values over the chosen period; the AUC or change scores. Where change scores are computed, subjects are often classified into 'responders/reactors' or 'non-responders/non-reactors' based on the size of the increase between the waking sample and the one taken subsequently (with mean increases of 2.49 nMol/l or greater often cited as being evidence of 'reactivity/response' (Wust et al., 2000; Kunz-Ebrecht et al., 2004).

Conversely, measures of the diurnal pattern of cortisol production necessarily require samples to be collected on several occasions during one or more days. These values are then used to determine the slope or pattern of diurnal change (Sephton et al., 2000; Vedhara et al., 2003). This is typically conducted using regression models. The level of cortisol is regressed on the time of collection to give a slope parameter. Because levels decline over the course of the day, the slopes are negative and so higher values (i.e. those closer to zero) are indicative of more abnormal diurnal patterns and lower values (i.e. those further away from zero) are indicative of more normal diurnal patterns.

1.2. Relationship between cortisol indices and measures of psychological functioning or clinical outcomes

In addition to there being variation in the approaches taken to measuring cortisol, it is clear that there is a lack of clarity regarding the relationship between these cortisol indices and measures of psychological functioning and/or clinical outcomes. This occurs at two levels. First, all of the indices described above have been shown to have inconsistent relationships with measures of psychological functioning (Vedhara et al., 2003). For example, while several investigators have reported evidence of increased cortisol levels in populations reporting elevated levels of stress (both acute and chronic) (Schulz et al., 1998; Melamed et al., 1999; Vedhara et al., 1999), there are many published studies that have failed to replicate this association (Al'Absi et al., 1997;

Marshall et al., 1998). This is also true of the evidence base pertaining to measures of the early morning peak and diurnal cortisol rhythm. With regard to the former, although several studies have shown that the early morning peak is greater in individuals reporting higher levels of stress or depressive symptoms (Schulz et al., 1998; Pruessner et al., 2003b); other studies have found highly stressed populations to exhibit lower early morning peaks or blunted responses (Prussner et al., 1999; Yang et al., 2001). With regard to diurnal rhythm, investigators have suggested that abnormal patterns are evident in people reporting greater distress; in women reporting less functional relationships (defined as the extent to which the participant reported positive feelings about relationships and felt able to utilise them for support and comfort) and they have also been found to be predictive of mortality in women with breast cancer (Vedhara et al., 2003; Adam and Gunnar, 2001; Sephton et al., 2000). However, other studies have shown that abnormal patterns are related to more positive outcomes such as less distress and fewer upper respiratory illnesses and symptoms (Smyth et al., 1997; Edwards et al., 2003). It is also worthnoting that this literature is further limited by a preponderance of research examining the relationship between cortisol and state measures of mood, and relatively little enquiry into the nature and/or direction of the relationship between cortisol and trait measures of psychological functioning.

The second area of uncertainty concerns the relationship *between* these various cortisol indices. It is clear that, despite reflecting different aspects of HPA function (e.g. basal activity versus reactivity), these indices are often used interchangeably. In addition, because few studies have been conducted in which more than one index is measured in the same population, the nature and the direction of the relationships, both between different cortisol indices, and also between these indices and the chosen outcome or dependent variable, remains unclear.

Where such relationships have been examined, an inconsistent relationship emerges. For example, Edwards and colleagues (2003) conducted a study examining the relationship between cortisol, perceived stress and upper respiratory symptoms. Their cortisol assessments included a measure of the early morning peak, absolute levels produced over 12-h periods and the diurnal cortisol rhythm. They observed that while the diurnal rhythm was related to the number of symptoms, the early morning peak and absolute levels of cortisol over a 12-h period were not. Similarly, when examining relationships with their measure of stress, they reported that no relationship was evident between stress levels and the diurnal cortisol rhythm or absolute cortisol levels; but that there was a positive relationship between the early morning peak measure and stress (i.e. individuals reporting greater stress had higher levels of cortisol post-waking).

Similar inconsistency was evident in a study reported by Porter and colleagues (2003) conducted with breast cancer survivors and women without a history of breast cancer. All women were attending for routine mammography, and salivary cortisol was measured the month before, the day before, the day of, and the day after the mammogram. Four cortisol measures were computed: mean baseline cortisol (the mean of the samples collected over 3 days, 1 month before mammography); diurnal rhythm (the diurnal slope of these 3 days, 1 month before mammography); the early morning peak (the mean of cortisol samples collected on waking, and 30 and 60 min post-waking) and cortisol reactivity (change scores reflecting the difference between mean cortisol levels 1 month before mammography and mean levels observed the day before, the day of, and the day after mammography). The results revealed that, while breast cancer survivors differed significantly from control women on the measures of mean baseline cortisol levels and cortisol reactivity, no group differences were evident on the measures of early morning peak or diurnal cortisol rhythm.

It is clear from this overview that several areas of uncertainty exist in this literature. The present study was designed to examine the following issues. First, what is the nature and direction of the relationship between four routinely used, but differing indices of cortisol production, i.e. AUC_g, AUC_i, the early morning peak and diurnal cortisol rhythm? Second, are these cortisol indices related to both state and trait measures of psychosocial functioning. Third, are the relationships between psychosocial functioning and these differing cortisol indices consistent? Fourth, are these relationships influenced by the clinical status of the participant, i.e. are similar relationships evident in both a clinical and non-clinical group? The population selected for study were women with breast cancer and community controls. This population were considered appropriate as much of the early work on diurnal cortisol rhythm, in particular, has been conducted in this clinical group (Sephton et al., 2000; Porter et al., 2003). Furthermore, the clinical significance of research with this group is underscored by evidence that patterns of cortisol production may not only reflect levels of distress,

but may also be related to mortality in women with breast cancer (Sephton et al., 2003).

2. Methods

2.1. Participants and recruitment

Newly diagnosed breast cancer patients were selected and invited to participate in a longitudinal study about relationships and adaptation to cancer. Both patients and their partners were recruited for the main study as it was concerned with how both members of the dyad respond to the disease and how their reactions affect each other. However, only data from patients and control women were examined for the present manuscript.

Participants were recruited from five hospitals in the northern part of the Netherlands. Inclusion criteria for the study were: (1) patients' age between 30 and 75 years old; (2) survival prognosis of at least 15 months; (3) living with a spouse; (4) no previous cancer history for patient nor spouse. All eligible couples were informed about the study by specialist breast cancer nurses. Details of couples who consented to participate were then sent to the research team who initiated contact and entry into the study.

Healthy control couples were selected from random samples of the register office of several townships in the same region of the participating hospitals and were sent a participation form. Control couples were matched individually to patient couples as soon as informed consent was received by each patient couple. Control couples were matched to patient couples on both the age of the patient and the region in which they resided. The only exclusion criterion for control couples was a history of cancer for either partner. Of the 64 control women who agreed to participate, cortisol data were available for 59 control women (two women refused to provide samples, the remaining three collected samples greater than 30 min outside of the allotted sample collection times and their data were, therefore, excluded). Control women who contributed cortisol data did not differ significantly from women who did not, on any of the demographic or psychological measures.

Of the 364 eligible patient couples, 284 couples considered participation, i.e. they took away written information about the study. All couples were given 2 weeks to consider whether or not to participate. Informed consent was obtained from 92 couples (25% response rate). The main reason given for non-participation was the perception that the burden of the study was too great (31%). A further 28% of couples indicated that they had no interest in the research; 15% stated that their decision not to participate was due to the unwillingness of the spouse, and 10% of couples indicated that they wanted not to dwell on the diagnosis of cancer. The remaining 16% of couples gave a variety of other reasons for not participating in the study. It should be noted, however, that although the recruitment rate of 25% is modest, 94% of couples completed all phases of the study. Furthermore, participating patients did not differ from non-participants with respect to age and region. Of the 92 couples who participated, cortisol data were collected from 85 patients (seven women refused to provide samples). Patients who contributed cortisol data did not differ significantly from patients who did not, on any of the demographic, clinical or psychological measures.

Clinical and demographic characteristics of the patients and control women who participated in the study and for whom complete data were available are presented in Table 1.

2.2. Measures

2.2.1. Salivary cortisol

Saliva for the cortisol assays was collected using salivettes (Starsedt) containing a piece of absorbent gauze. For patients, saliva collection occurred a mean of 6.29 days after questionnaire completion and for control women it occurred a mean of 5.11 days after questionnaire completion. Individuals were asked to place this gauze in their mouths and roll it around until it was saturated. The gauze was then placed back into the salivette. Participants were asked to collect eight saliva samples over the course of two consecutive days: (1) directly after waking (before breakfast); (2) 30 min later; (3) before lunch; (4) late at night (at least 2 h after evening meal). For all samples, participants were asked to refrain from eating or drinking within 30 min of providing the sample. Participants were also asked to note on each salivette the precise time of sample collection. Collection times that were greater than 30 min outside of the specified time were excluded from the analysis. For patients this resulted in no exclusions. However, the data from three control women were excluded as they failed to collect their samples within the allotted times. Samples were kept refrigerated and then sent to the central laboratory of the University Hospital of Groningen. Upon arrival in the laboratory, the samples were frozen.

On the day of the radioimmunoassay, the salivette was thawed at room temperature and spun down at 3000 rpm (1900*g*) for 15 min.

Table 1 Descriptive characteristics of patients and female controls.

	Patients ($N=85$)	Female controls ($N=59$)	p
Mean age	52.5 (SD 9.3)	53.1 (SD 10.0)	ns
Education			
Primary	8	3	ns
Lower vocational/secondary	30	24	
Middle vocational/secondary	29	1	
Higher vocational/university	25	22	
Employed, yes	44	32	ns
Initial treatment			
Only surgery	9		
Surgery + radiotherapy	32		
Surgery + chemotherapy	31		
Surgery + radio + chemotherapy	1		
Surgery + hormonal therapy	4		
Surgery + radio + hormone therapy	7		
Surgery + other adjuvant treatment	8		
Treatment at time of study			
No treatment	29		
Radiotherapy	17		
Chemotherapy	24		
Hormonal therapy	8		
Radiotherapy + hormonal therapy	3		
Chemotherapy + hormonal therapy	3		
Other adjuvant treatment	1		

Saliva was added in duplicate to tubes which contained buffer (0.1 M Tris–NaCl-globuline pH 8.0), cortisol antibody and radioactive tracer (1,2, 6,7-^{3}H cortisol, 250 Bq/100 μl by Perkin Elmer). The tubes were then mixed and left to incubate for 30 min at 60 °C followed by a 2 h rest on ice. Before another incubation period of 20 min on ice, 0.2 ml of charcoal suspension was added to each of the samples and standards. All tubes were spun down at 3000*g* and 4 °C for 15 min. Supernatants were pipetted off and placed into pony vials containing 1 ml UltimaGold XR (Perkin-Elmer) before counting in a Beta liquid scintillation counter (Packard). Results were extrapolated from standard curves and concentrations from each of the sampling times expressed as nmol/l. The intra-assay variation coefficients of the normal salivettes was ±8.2% by a concentration of 1.5 nmol/l ±4.1% by a concentration of 15 nmol/l and ±5.4% by a concentration of 30 nmol/l. The inter-assay variation coefficients were 12.6, 5.6 and 6.0%, respectively. The limit of detection was 0.9 nmol/l.

The cortisol data were treated in four ways. The first two involved estimations of the AUC. Using the formulae outlined by Pruessner and colleagues (2003a), both AUC_g and AUC_i were calculated. In accordance with the interpretation offered by the authors, the AUC_g measure (defined as 'AUC with respect to ground') provides information on total hormonal output, thus, the basal activity of the HPA axis; while the AUC_i measure (defined as 'AUC with respect to increase') provides information on the reactivity of the system. As a result of exclusions and missing data, complete AUC_g and AUC_i data were available for 76 patients and 55 control women.

The calculation and difference between AUC_i and AUC_g is illustrated in Fig. 1 using one case from the present data. In addition, Fig. 2a–d illustrates four simulated scenarios in which the two AUC measures are calculated to demonstrate the differing information that can be captured by each measure. Fig. 2a shows a large reduction in cortisol levels over the course of the day which is represented by AUC_i. However, total levels of cortisol, as represented by AUC_g, are relatively low. In comparison, Fig. 2b shows a small change in cortisol over the day (represented by AUC_i), while total levels of cortisol production (represented by AUC_g) are high, because levels are high throughout the day. In this way, the AUC_g measure in Fig. 2a and b are similar, while the AUC_i measures are very different. In Fig. 2c, the change over the day is similar to Fig. 2b, so the AUC_i will be similar. However, both the start and end points are very different, thus, the AUC_g measure will be very different. Finally, Fig. 2d shows a large change over the day. Thus, AUC_i will be similar to that observed in Fig. 2a. However,

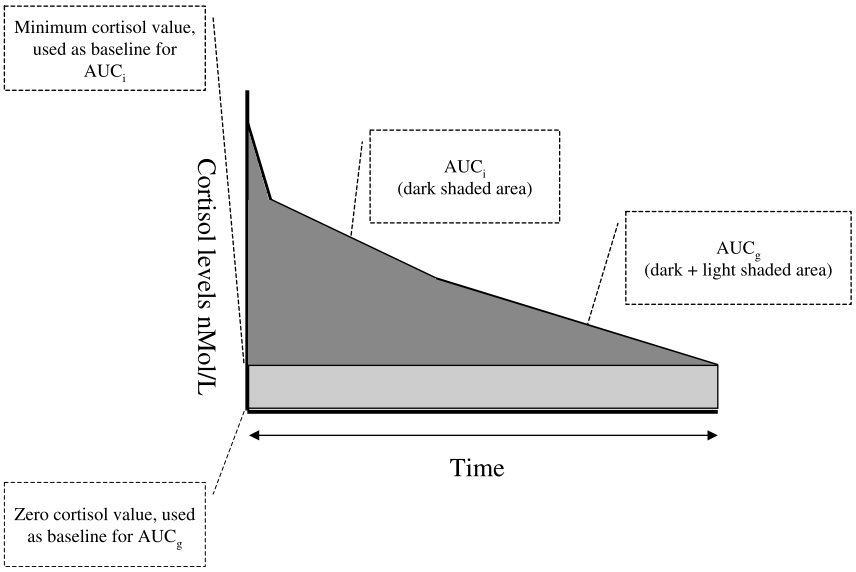

Figure 1 Illustration of the difference between AUC_i and AUC_g.

because the minimum value is higher, the AUC_g will be higher than that observed in Fig. 2a.

The early morning peak and diurnal pattern of cortisol production were also computed. For the former, the second cortisol measure (sample taken 30 min after waking) was taken away from the first cortisol measure (sample taken on waking). This provided us with a measure of the absolute change in cortisol levels in the first 30 min following waking. This measure was calculated for each day on which saliva was collected. Early morning peak data were available for 78 patients and 57 control women. In order to determine the diurnal pattern of cortisol, the cortisol values were transformed using a natural log, which normalised the distribution and linearised the change over the day. However, the second sample of each day (i.e. 30 min post-waking) was omitted because the inclusion of the early morning peak would have distorted the determination of the slope. A linear slope was calculated for every individual who had all three measures (i.e. waking sample, 11–1 p.m.

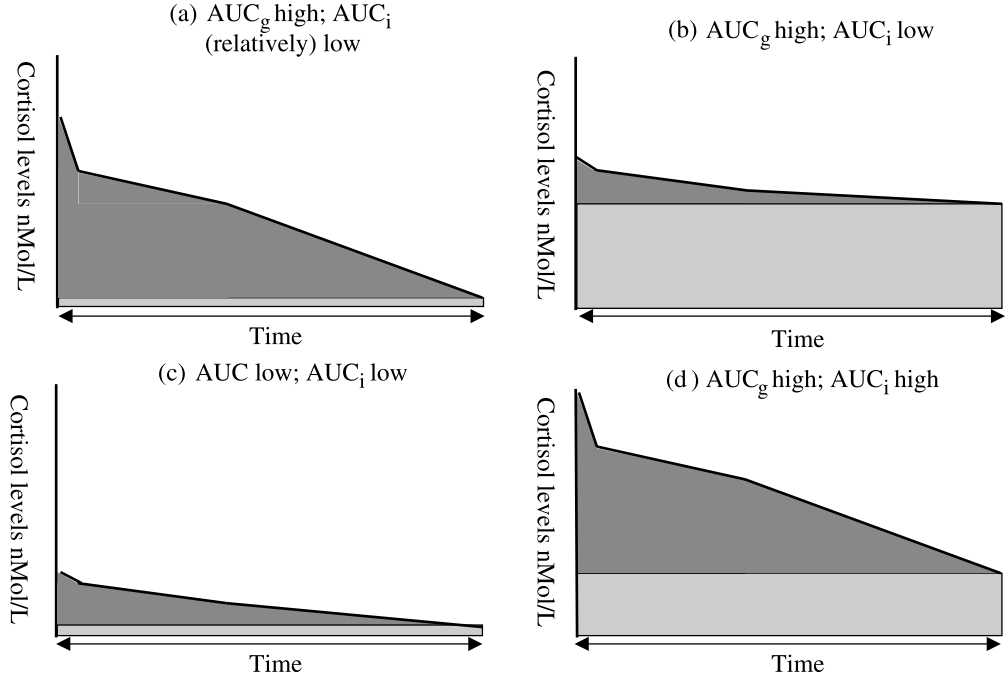

Figure 2 Four different scenarios illustrating the difference between AUC_g and AUC_i.

sample and 8–10 p.m. sample) on both days. This resulted in the exclusion of only four control women and nine patients, i.e. slope data were available for 55 controls and 76 patients. The slope was calculated by regressing the cortisol level onto the time of the sample, with the first sampling occasion treated as time = 0, and later times being the number of hours after this time. The resulting slope measure was the expected amount that logged cortisol levels dropped per hour over the day, for each individual. As with previous research, higher values, or those closer to zero, were indicative of more abnormal diurnal patterns and lower values, or those further away from zero, were indicative of more normal diurnal patterns. As a result of exclusions and missing data, complete slope data were available for 76 patients and 55 control women.

2.3. Psychosocial measures

The psychosocial measures selected for inclusion in this study reflected both state and trait measures of psychosocial functioning. The state measures included measures of mood that have been widely investigated in the relationship between cortisol and psychosocial functioning, i.e. distress, anxiety and depression. The selection of trait measures was also guided by the existing literature. For example, of the few investigations that have adopted trait measures previously, measures of personality such as neuroticism have been the most widely investigated. Thus, both neuroticism and extraversion were examined. Similarly, there is considerable body of work which has identified aspects of coping style and social support as being central to individuals' adjustment to a range of chronic diseases, including breast disease. In the present study, the dimensions of coping and support highlighted for study were mastery and marital satisfaction, respectively, as they were central to the wider aims of the research which seeks to explore how the quality of marital relationships influences adaptation to cancer.

Anxiety and depression: were measured using the Hospital Anxiety and Depression Scale (HADS: Zigmond and Snaith, 1983). The HADS is a 14-item self-report scale and contains two 7-item scales: one for anxiety and one for depression both with a score range of 0–21. The scale has been used widely and validated for use with Dutch populations (Spinhoven et al., 1997). In the present study, the internal reliability (coefficient alpha) of the anxiety scale was .94 for the patients and .84 for the female controls, for the depression scale subsequently .95 and .72.

2.3.1. Psychological distress

This was measured using the General Health Questionnaire, (GHQ: Goldberg and Williams, 1988). This scale measures general psychological distress, as opposed to the more specific components of anxiety and depression captured by the HADS. In addition, the GHQ includes somatic symptoms which are not covered by the HADS. The 12-item version of the GHQ was used with a score range of 0–36. A salient point of the GHQ is that the respondent is his or her own reference. With each statement, the actual status of the respondent (over the past four weeks) is compared with his or her 'normal' status by one of four responses. For this study the internal reliability of the GHQ was .86 for the patients and .87 for the female controls.

2.3.2. Neuroticism and extraversion

These were assessed by means of a subscale of the short form of the Eysenck Personality Questionnaire-Revised (EPQR-S: Eysenck et al., 1985). This scale has also been validated in Dutch populations (Sanderman et al., 1991). Both scales have 12 items, with higher scores indicate greater neuroticism or extraversion. The scales have good psychometric properties: the α for the neuroticism scale was .83 for the patients and .84 for the female controls, for the extraversion scale subsequently .83 and .76.

2.3.3. Marital satisfaction

This was assessed using the 10-item Maudsley Marital Questionnaire (MMQ: Arrindell et al., 1983) which has been used previously in Dutch populations (Kempen et al., 2000). Higher scores on this scale indicate greater marital satisfaction. For this study the internal reliability of the MMQ was .91 for the patients and .87 for the female controls.

2.3.4. Mastery

Pearlin and Schooler (1978) Mastery scale was used to measure mastery, i.e. the extent to which people believe that their behaviour matters for the events that occur in their environment. This scale theoretically ranges from 7 (low mastery) to 35 (high mastery). Cronbach's alpha is .74 for patients and .69 for female controls.

2.4. Procedure

The baseline measurement took place 3 months after diagnosis. Research assistants interviewed patient and spouse separately at home using structured interview questionnaires. Socio-demographic characteristics of all subjects and medical characteristics of the patients were assessed during

Table 2 Pearson's product moment correlations between individual cortisol indices over day 1 and day 2.

	Patients	Control women
Waking sample	0.690, $p<0.001$ ($n=78$)	0.555, $p<0.001$ ($n=57$)
30 min post waking	0.712, $p<0.001$ ($n=82$)	0.301, $p=0.023$ ($n=57$)
11–1 p.m.	0.467, $p<0.001$ ($n=81$)	0.484, $p<0.001$ ($n=57$)
8–10 p.m.	0.552, $p<0.001$ ($n=81$)	0.518, $p<0.001$ ($n=55$)
Early morning peak	0.914, $p<0.001$ ($n=78$)	0.349, $p=0.007$ ($n=58$)
Diurnal cortisol rhythm	0.521, $p<0.001$ ($n=76$)	0.383, $p=0.004$ ($n=55$)
AUC_g	0.706, $p<0.001$ ($n=76$)	0.674, $p<0.001$ ($n=55$)
AUC_i	0.355, $p=0.002$ ($n=76$)	0.220, $p=0.107$ ($n=55$)

these face-to-face interviews. During the interview, patients and spouses also completed a self-report questionnaire containing the psychological indices outlined above.

3. Results

3.1. Correlations between day 1 and day 2 cortisol measures

Pearson's product moment correlations were computed for each of the individual cortisol measures (i.e. waking sample, 30 min post-waking, 11–1 p.m. and 8–10 p.m.) and the computed cortisol indices (i.e. AUC_g, AUC_i, diurnal cortisol and early morning peak) over the 2 days of sample collection. The results revealed a high degree of correlation for all of the measures over the 2 days, for both patients and control women (see Table 2). The only exception was the correlation between the AUC_i index on days 1 and 2 for control women which failed to attain significance. In view of these significant correlations, mean cortisol scores were used for all subsequent analyses.

3.2. Correlations between different cortisol indices

Further correlations were computed to examine the nature of the relationship between the four derived cortisol indices in the patient and control samples. The results revealed that, for patients, the early morning peak correlated positively with AUC_g, but not AUC_i; and that a significant negative correlation existed with diurnal cortisol rhythm. AUC_g was not found to correlate significantly with any other index, while AUC_i only correlated negatively with diurnal cortisol rhythm.

For control women, some similar relationships were evident with the relationship between early morning peak and AUC_g approaching significance, but no significant associations evident with AUC_i and diurnal cortisol. However, AUC_g and diurnal cortisol were significantly positively correlated, while AUC_i and diurnal cortisol were significantly negatively correlated (Table 3).

3.3. Differences in cortisol and psychosocial functioning between patient and control women

Table 4 presents the mean scores for all four cortisol indices and all the measures of psychosocial functioning for patients and control women. A series of *t*-tests revealed that the two groups only differed significantly on the measure of distress (greater distress in patients than controls).

Table 3 Pearson's product moment correlations between different cortisol indices (mean of days 1 and 2).

		Early morning peak	AUC_g	AUC_i
AUC_g	Patients	0.361, $p<.001$		
	Controls	0.234, $p=0.080$		
AUC_i	Patients	0.111, $p=0.340$	0.091, $p=0.431$	
	Controls	0.033, $p=0.809$	−0.331, $p=0.012$	
Diurnal cortisol rhythm	Patients	−0.263, $p=0.022$	−0.032, $p=0.783$	−0.769, $p<0.0001$
	Controls	−0.130, $p=0.345$	0.419, $p<.001$	−0.771, $p<0.001$

Table 4 *t*-Tests comparing patients and controls on cortisol and psychosocial measures.

	Group	Mean	SD	Difference (95% CIs)	p
AUC_g (mean of days 1 and 2)	Control	21.22	5.06	1.065 (−2.75, 1.46)	0.546
	Patient	20.58	6.81		
AUC_i (mean of days 1 and 2)	Control	12.71	4.85	0.801 (−1.36, 1.81)	0.777
	Patient	12.94	4.45		
Early morning peak (mean of days 1 and 2)	Control	7.01	6.24	4.132 (−4.06, 12.29)	0.322
	Patient	11.12	30.98		
Diurnal cortisol rhythm (mean of days 1 and 2)	Control	−0.134	0.035	0.007 (−0.014, 0.015)	0.882
	Patient	−0.133	0.045		
Anxiety	Control	5.53	3.775	0.09 (−1.06, 1.24)	0.875
	Patient	5.44	3.410		
Depression	Control	3.25	2.684	−0.88 (−2.01, 0.25)	0.125
	Patient	4.13	3.973		
Distress	Control	11.19	4.734	−3.53 (−5.32, −1.74)	<0.001
	Patient	14.72	6.004		
Neuroticism	Control	3.95	3.248	−0.35 (−1.40, 0.70)	0.509
	Patient	4.30	3.220		
Extraversion	Control	7.70	2.734	0.22 (−0.75, 1.19)	0.657
	Patient	7.49	3.149		
MMQ	Control	66.78	9.011	0.29 (−3.01, 3.6)	0.864
	Patient	66.49	11.018		
Personal mastery	Control	24.43	3.950	0.15 (−1.23, 1.54)	0.830
	Patient	24.27	4.490		

3.4. Correlations between psychosocial variables and the four cortisol indices

Pearson's correlations were then computed to examine the strength and direction of any relationships between the cortisol indices and the selected psychosocial variables. Separate correlations were computed for patients and controls. The results, in Table 5, revealed that, for patients, significant correlations were evident between the mean early morning peak measure and neuroticism (positive correlation) and personal mastery (negative correlation). For control women, significant correlations were evident between AUC_g and personal mastery (negative correlation); AUC_i and distress (positive correlation) and diurnal cortisol and marital satisfaction (positive correlation).

3.5. Psychosocial predictors of cortisol indices

A series of linear regressions were then conducted to examine the extent to which the selected psychosocial variables predicted the cortisol outcomes and to examine the role of the mood measures (i.e. anxiety, depression and distress) separately from the trait-like dimensions (i.e. neuroticism, extraversion, MMQ and personal mastery. For all analyses, mean cortisol measures were used. The results revealed that none of the models for either state or trait measures achieved significance, with the exception of the model examining the role of trait measures in predicting early morning peak in patients ($R_2 = 0.130$, $p = 0.042$), in which neuroticism emerging as a significant predictor (standardised $\beta = -0.333$; $t = 2.758$; $p = 0.007$).

4. Discussion

The present study was designed to examine the relationship between measures of psychological functioning and four commonly used indices of cortisol production in women diagnosed with breast cancer and a control population. The study aimed to (i) examine the nature and direction of the

Table 5 Pearson's product moment correlations between cortisol indices and measures of psychosocial functioning.

	Group	AUC_g	AUC_i	Early morning peak	Diurnal cortisol rhythm
HADS anxiety	Control	−0.026	0.115	0.177	−0.175
	Patient	0.077	−0.033	−0.079	0.110
HADS depression	Control	0.043	0.104	0.048	−0.133
	Patient	0.195	0.016	0.072	−0.053
Distress	Control	−0.129	0.275*	0.125	−0.164
	Patient	0.000	−0.123	0.159	0.166
Neuroticism	Control	−0.021	0.135	0.060	−0.112
	Patient	0.022	−0.166	0.380**	0.029
Extraversion	Control	−0.063	0.069	−0.143	−0.073
	Patient	0.159	0.200	−0.117	−0.100
Marital satisfaction	Control	0.179	−0.252	0.104	0.309*
	Patient	−0.092	0.032	−0.120	0.015
Personal mastery	Control	−0.288*	0.037	−0.153	−0.115
	Patient	−0.209	0.126	−0.271*	−0.028

*$p<0.05$; **$p<0.01$.

relationship between the four indices, i.e. AUC_g, AUC_i, the early morning peak and diurnal cortisol rhythm; (ii) explore whether the cortisol indices were related to both state and trait measures of psychosocial functioning; (iii) whether the relationships between psychosocial functioning and these differing cortisol indices were consistent and finally (iv) examine whether these relationships were influenced by the clinical status of the participant?

With regard to the first issue, the four derived cortisol measures each represent differing features of cortisol production. The diurnal cortisol rhythm represents the pattern of cortisol production over time. The early morning peak reflects the reactivity of the HPA axis in response to waking. AUC_i reflects reactivity of the HPA axis over the day, while AUC_g reflects the basal activity of the HPA axis over the day. Nonetheless, the correlation analysis revealed the presence of some significant correlations between these indices. Significant negative correlations were evident between AUC_i and diurnal cortisol (i.e. the greater the daily reactivity, the more normal the diurnal cortisol rhythm) and significant positive correlations between the early morning peak and AUC_g (i.e. the greater the cortisol response to waking, the higher the basal cortisol levels over the course of the day). This pattern of results was evident for both groups. Other associations that were observed, but not found in both groups, were a negative correlation between early morning peak and diurnal cortisol for patients, but not controls (indicating that greater cortisol responses to waking were associated with more normal patterns of cortisol production). While for controls, but not patients, AUC_g was negatively correlated with AUC_i (higher basal levels of cortisol were associated with greater reactivity of the HPA axis) and positively correlated with diurnal cortisol (higher levels of basal cortisol were associated with more abnormal patterns of cortisol production).

These results underscore the differing nature of each of the selected cortisol indices. Even where significant correlations were evident, the variables captured divergent features of HPA axis functioning. This is most clearly illustrated with the two indices reflecting reactivity of the axis, i.e. the early morning peak and AUC_i. The independence of these indices was evident at several levels. For example, the early morning peak and AUC_i measures were found not to correlate significantly in either the patient or control samples. Furthermore, high levels of reactivity over the day (as expressed by the AUC_i measure) were associated with a more normal pattern of cortisol production, a finding which previous research would suggest may be advantageous to women with breast disease (Sephton et al., 2003). Indeed, the potentially adaptive nature of high levels of reactivity over the day is further supported by the negative correlation between AUC_i and AUC_g in control women, a finding indicating that greater reactivity of the axis was associated with overall lower basal levels of cortisol. Conversely, high levels of reactivity in response to waking (as reflected in the early morning peak measure) was associated with overall higher basal levels of cortisol, a finding which previous research would suggest may be a disadvantage for health in both clinical and non-

clinical groups (Andersen, 2002; Andersen et al., 1994). Together, these results suggest that AUC_i and the early morning peak reflect different features of HPA axis reactivity and that the former may be related to advantageous outcomes, while the latter may be associated with more adverse effects.

A further observation from these results concerns the fact that no between group differences were evident for any of the cortisol measures. This is not in keeping with some of the other published literature in this field which has shown, for example, the presence of flatter diurnal rhythms and higher mean cortisol levels in women with breast cancer, compared with controls (e.g. Abercrombie et al., 2004). The differences between these investigations may reflect differences in the disease stage of the patients and/or in their treatment modalities.

The second and third aims of this investigation were concerned with the nature of the relationship between the four cortisol indices and the selected psychosocial measures. Two main observations can be made. Firstly, it is clear that there was only limited evidence of significant relationships between the cortisol indices and psychosocial measures: only five of the possible correlations emerged as significant (see Table 5) and the only regression model to achieve significance was the prediction of the early morning peak in patients by neuroticism. Secondly, where significant relationships did emerge, there was a trend for trait measures to achieve prominence over mood measures. In particular, four out of five of the correlations were between trait measures and cortisol indices and, as already described, the only significant regression model also involved a trait measure.

The apparent absence of relationships between mood measures and cortisol, and conversely, the apparent presence of some relationships between trait measures and cortisol are worthy of comment. With regard to the former, the paucity of reliable relationships between cortisol indices and measures of mood has been observed by several other groups (e.g. Al'Absi et al., 1997; Marshall et al., 1998; Edwards et al., 2003; Porter et al., 2003). However, it remains unclear whether such findings highlight a genuine absence of reliable relationships between mood and cortisol, or reflect methodological difficulties. It would appear reasonable to expect that at least some of the inconsistency in the evidence is due to variations in the conceptualisation of both cortisol and mood between different studies. With regard to the conceptualisation of cortisol, the present study has illustrated how four commonly used cortisol indices diverge in their relationships not only with each other, but also with selected psychosocial parameters. Indeed, these results emphasise the importance of not using different indices of cortisol interchangeably. Similarly, the recent work of Polk and colleagues illustrates the potentially confounding effects of differing conceptualisations of mood (Polk et al., 2005a,b). They distinguished between positive and negative mood, as well as state and trait measures of mood. Trait measures of mood were more closely related to cortisol than state measures, and positive mood states were found to influence levels and patterns of cortisol production. Taken together, these findings highlight that much greater precision is required in the conceptualisation of both mood and cortisol if we are to be able to achieve a clearer understanding of the relationship between these variables.

With regard to relationships between trait measures and the cortisol indices, the measures of mastery and neuroticism are worthy of further comment. In both patients and control women, greater mastery was associated with more adaptive features of cortisol production, including lower basal cortisol levels over the day (i.e. AUC_g) and lower cortisol responses to waking (i.e. early morning peak). The literature on the HPA axis activity and mastery is, however, limited. Indeed, at the time of conducting the present study, we were unable to locate any other publications in this area. Thus, while it is not possible to examine the extent to which our findings concur with those of other groups, the present data suggest that mastery may be a trait worthy of further investigation.

In contrast, neuroticism is among the most widely investigated trait measures in this area (Roy, 1996; Schommer et al., 1999; Hanson et al., 2000; Habra et al., 2003; Zobel et al., 2004). Although, here too, the evidence is equivocal. The present study revealed the presence of a relationship between cortisol and neurotcism, this was, however, only apparent in the patient sample and with a cortisol measure reflecting reactivity of the axis (i.e. the early morning peak). These findings can be seen to be in keeping with other research, such as the work of Habra and colleagues, who also observed an association between neuroticism and an index of HPA reactivity, as measured by cortisol reactivity to a mental arithmetic task (Habra et al., 2003). Similarly, Zobel et al. (2004) reported an association between neuroticism and reactivity of the HPA axis, as measured by cortisol responses to the dexamethasone suppression test.

The final aim of this research which was concerned with the consistency of the relationships

evident for patients and control women. Considerable variability was evident not only between the four measures of cortisol production, but also the direction and nature of the relationships with the psychosocial measures. The causes of these differences is beyond the scope of the present work, but they may be related to the clinical status of the patients, with the disease and/or its treatment serving to distort the relationships between the four cortisol measures. Or indeed, the differences may be due to disparities in the psychosocial functioning of the two groups, as there was evidence of greater distress in the patients compared with the control women.

Finally, it is important to highlight some methodological issues in the present study which may, themselves, have influenced the results obtained and the conclusions drawn. First, the effects of poor compliance with sampling protocols are well known (Broderick et al., 2004). We sought, therefore, to maximise compliance by requesting that participants indicate the precise times at which they collected their samples and excluded data from those individuals whose samples were collected greater than 30 min outside the allotted time frames. The validity of this approach is, however, predicated on participants recording their sample collection times accurately. It remains probable that, for some participants, these data were not recorded accurately and this may, in turn, have affected our findings. Second, we did not take into account the potentially confounding effects of disease stage or initial and concurrent treatments in our analyses. Although the literature suggests that the effects of treatments, such as chemotherapy, on cortisol are marginal (Kailajarvi et al., 2000), it is possible that disease and treatment factors affected our data. Third, it should be acknowledged that the validity of our findings is influenced to a large extent by the reliability of our cortisol data. As has been noted previously, the propensity for variability in cortisol levels is considerable both between and within individuals (Bohnen et al., 1991; Cummins and Gevirtz, 1993). While, our analyses suggested the presence of some consistency over the two sampling days (see Table 2), it was also clear that this consistency was not achieved for the AUC_i measure and the size of some of the other correlations was modest. Moderate reliability in the cortisol data could clearly have influenced the reliability of the associations observed not only between the four cortisol indices, but also with the selected psychosocial parameters. Finally, the study necessarily limited by its cross-sectional nature which precluded analyses into how the measures of psychosocial functioning and cortisol were related to clinical outcomes.

In summary, the present study has illustrated the divergent nature of the four cortisol indices; revealed the presence of some significant relationships between the psychosocial measures and the cortisol indices; but highlighted inconsistencies in the relationships evident for patients and those observed for control women. Despite the methodological limitations acknowledged above, the results can be seen to contribute to the debate on how psychosocial factors are related to cortisol and the methodologies used to investigate these relationships.

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